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CESIUM-SODIUM INTERACTIONS ON X-RAY DEPOLARIZATION OF MUSCLE FIBERS

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### Introduction:

In a previous publication (1), we have shown that X-irradiation of frog sartorius muscle fibers produces a depolarization whose magnitude depends on the sodium and potassium ion concentrations in the bathing fluid. However, the fractional depolarization is independent of external potassium ion concentration, above 1 mM, and proportional to the external sodium ion concentration. We concluded that the X-ray induced depolarization is primarily the result of an increase in sodium permeability, with no increase in potassium permeability. Several investigators have reported a relationship between calculated alkali cation radius and rate of penetration of non-irradiated muscle fiber membrane (2,3). We have therefore studied the effects produced on X-ray induced depolarization of frog sartorius fibers by adding various concentrations of cesium ions to the bathing solution in place of potassium ions.

### Methods:

Sartorius muscles ranging from 90 to 100 mg. fresh weight were dissected carefully so as to leave the lower side as free as possible from connective tissue. One muscle from each frog was used for irradiation, one as a control. Ten frogs were used for each solution. The muscles were mounted in a special holder at 120% of their resting length by a micrometer system. Muscles were equilibrated for 60 minutes in the experimental solution prior to micro-electrode penetration. The volume of bathing solution used at each concentration was 500 ml. The solution was not "aerated" with the usual  $O_2$ - $CO_2$  gas mixture in order to minimize mechanical damage to the surface of the muscle cells. All experiments were carried out

at  $25.0 \pm 0.5^{\circ}\text{C}$  and pH 7.3.

Membrane potentials were measured with 3 M KCl-filled micro-pipette electrodes having resistances between 10 and 12 megohms. The electrical measurements were made with conventional equipment for this work. Only potentials that developed instantaneously with penetration of the muscle cell by the microelectrode were recorded and used in the data. Each plotted point in the figures represents an average of potential measurements on 80 cells. The standard error of the mean is less than 0.8 mV.

Muscles were irradiated while mounted in the chamber at 6,000 r/min. to a total dose of 100 Kr using a G. E. Maxitron X-ray machine at 300 KVP, 20 ma, with 0.2 mm Al filter. Ringer's solution of standard composition (2.5 mM KCl, 1.89 mM  $\text{CaCl}_2$ , 112 mM NaCl, 2.5 mM  $\text{Na}_2\text{HPO}_4$ , 0.5 mM  $\text{NaH}_2\text{PO}_4$ ) was modified for these experiments by replacing the potassium chloride by cesium chloride over a range of concentrations, ranging from 10 to 80 mM Cs.

#### Results:

After 4-6 hours of soaking, at concentrations of CsCl above 10 mM, a high rate of breakage of microelectrodes and unusual difficulty in obtaining resting potential measurements were encountered. At 80 mM Cs, it is observed that a significant number of muscle cells either develop no membrane potentials or very low potentials. Spontaneous activity was frequently observed under these conditions. Such effects were not observed in the corresponding high-potassium solution studied in our previous work (1). These qualitative observations cast some doubt on the physiological acceptability of cesium as a "substitute" for potassium ions.

Table 1 summarizes the results obtained in 10 mM Cs, at two sodium concentrations, normal (112 mM NaCl) and half normal (56 mM NaCl, 56 mM choline chloride). The average resting membrane potential in standard Ringer's solution with no cesium under these conditions is  $89 \pm 0.5$  mV. Table 1 therefore indicates that in the absence of irradiation, 10 mM Cs (112 mM Na) depolarizes by approximately 17 mV (membrane potential: 72 mV), but the degree of Cs depolarization depends on the external sodium concentration, being 29 mV (membrane potential: 61 mV) in 56 mM Na. Irradiation produces an additional depolarization. The fractional depolarization resulting from irradiation is proportional to the external sodium concentration as was observed in potassium experiments (1). Thus the ratio of control to irradiated potentials is 1.24 in normal sodium, 1.12-1.13 in half normal sodium (Table 1) compared to 1.25 and 1.13, respectively, found in 10 mM potassium (Ref. 1, Figure 2). It should also be noted in Table 1 that the potentials are constant with time after irradiation in both control and irradiated muscles. Table II and III show that the results obtained in 20, 50 and 80 mM Cs are approximately similar to those in 10 mM cesium, 112 mM Na. The measured potentials, at higher concentrations of Cs (see Table 3) were found to be time-dependent.

#### Discussion:

These results, summarized in Table 4, indicate that the fractional depolarization produced by irradiation is independent of external cesium concentration, and dependent on external sodium concentration. We conclude, just as in our previous work (1), that irradiation depolarization results from an increment of sodium

permeability which is constant for the period of experimentation and is not altered by the presence of external 10 mM Cs ions or external potassium ions (1). Consequently, these results also demonstrate that the potassium and cesium permeabilities are not changed by irradiation.

The increase in cesium depolarization with decreasing external sodium concentration (Table 1) as well as the apparent independence of cesium and irradiation in producing depolarization, indicate that the cesium depolarization observed in control fibers is not primarily due to an increase in sodium permeability. The results of the study of Na-dependence of the Cs depolarization (in 20 mM Cs external concentration) are summarized in Figure 1. The stability with time of the lowered potentials over the range from 10 to 20 mM cesium concentrations even after irradiation, suggests that cesium has not interfered with the ability of the sodium extrusion mechanisms to keep up with the increased sodium influx produced by irradiation. The sodium-dependence of the cesium depolarization is in sharp contrast to the sodium-independence shown by the depolarization resulting from increased external potassium in the absence of cesium (1). It is clear not only that cesium and potassium are not interchangeable so far as the membrane is concerned, but also that the membrane response to cesium is specifically different from its response to potassium ions. It is known that the latter approaches very closely a simple potassium concentration cell. The results reported here indicate that the cesium effect cannot be so simply explained. The direction of the sodium effect suggests a specific sodium-cesium interaction in the membrane mechanisms in such a way that sodium ions interfere with the permeation of cesium ions. The cells become



progressively depolarized with time by 80 mMCs; this decline in membrane potential is related to an increase of Cs uptake and to a progressive damage at the cell surface and its metabolism.

Summary and Abstract:

Cesium ions in the bathing fluid produce a depolarization of frog sartorius muscle fibers independent of the depolarization produced by irradiation. The fractional depolarization produced by X-rays is independent of external cesium concentration. However, the fractional depolarization is proportional to external sodium concentration and is therefore explained as the result of an increased sodium ion permeability. Cesium depolarization (20 mMCs) in non-irradiated fibers increases with decreasing sodium concentration, indicating a specific sodium-cesium interaction in the membrane. A simple concentration-cell potential explanation for the cesium effects is not yet adequate.

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Figure 1. - The membrane potential obtained in 20 mMOCs, at 5 sodium concentrations is plotted against time.

A. V: 112 mMNa; W: 97 mMNa; X: 83 mMNa; U: 56 mMNa;  
and Z: 31 mMNa.

B. The membrane potential obtained in 20 mMOCs is plotted against the external concentration of Na ions.

10 mMCs				
Time (hrs)	112 mMCs		56 mMCs	
	C(mV)	I(mV)	C(mV)	I(mV)
1	71.60 $\pm$ 0.60*	71.70 $\pm$ 0.57	60.4 $\pm$ 0.82	60.6 $\pm$ 0.56
2	71.65 $\pm$ 0.57	71.81 $\pm$ 0.49	60.1 $\pm$ 0.72	60.18 $\pm$ 0.50
3		Irradiation Period		Irradiation Period
4	72.30 $\pm$ 0.64	57.53 $\pm$ 0.70	60.8 $\pm$ 0.83	55.9 $\pm$ 0.66
5	71.55 $\pm$ 0.53	56.80 $\pm$ 0.75	61.6 $\pm$ 0.49	53.8 $\pm$ 0.43
6	71.57 $\pm$ 0.44	57.14 $\pm$ 0.63	61.5 $\pm$ 0.50	54.4 $\pm$ 0.52
7	71.60 $\pm$ 0.43	57.29 $\pm$ 0.58	61.5 $\pm$ 0.49	53.82 $\pm$ 0.58

\*S.E.M.

20 mMCs		
Time (hrs)	112 mMCs	
	C(mV)	I(mV)
1	60.64 $\pm$ 0.67	60.51 $\pm$ 0.76
2	60.42 $\pm$ 0.72	60.73 $\pm$ 0.73
3		Irradiation Period
4	60.95 $\pm$ 0.72	49.4 $\pm$ 0.45
5	60.0 $\pm$ 0.64	49.8 $\pm$ 0.42
6	59.9 $\pm$ 0.71	49.9 $\pm$ 0.63
7	60.0 $\pm$ 0.34	49.6 $\pm$ 0.69

Time (hrs)	50 mMCs, 112 mR/h		50 mMCs, 112 mR/h	
	C(mV)	I(mV)	C(mV)	I(mV)
1	52.4 $\pm$ 0.48	52.40 $\pm$ 0.56	51.4 $\pm$ 0.46	51.8 $\pm$ 0.39
2	52.9 $\pm$ 0.41	52.38 $\pm$ 0.45	49.9 $\pm$ 0.65	49.9 $\pm$ 0.41
3		Irradiation Period		Irradiation Period
4	52.65 $\pm$ 0.72	41.45 $\pm$ 0.94	49.8 $\pm$ 0.65	40.1 $\pm$ 0.61
5	52.78 $\pm$ 0.45	41.0 $\pm$ 0.48	49.0 $\pm$ 0.42	39.2 $\pm$ 0.60
6	53.1 $\pm$ 0.51	40.7 $\pm$ 0.53	47.7 $\pm$ 0.67	39.9 $\pm$ 0.70
7	52.9 $\pm$ 0.53	40.1 $\pm$ 0.45	48 $\pm$ 0.60	37.7 $\pm$ 0.80
8	52.8 $\pm$ 0.48	40.3 $\pm$ 0.63	46.7 $\pm$ 0.50	38.0 $\pm$ 0.90

10 mMCs		20 mMCs	50 mMCs	80 mMCs
112 mMNa	56 mMNa	112 mMNa	112 mMNa	112 mMNa
C/I	C/I	C/I	C/I	C/I
1.854	1.13	1.21	1.3	1.24

